Journal of Basrah Researches ((Sciences)) Vol. (47). No. 1 (2021)

Available online at: https://www.iasj.net/iasj/journal/260/issues

ISSN –1817 –2695

Received date: 10-11-2019 Accepted date: 5-1-2020 Available online date: 31-12-2021

EFFECT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY ON SOME ENZYMES AND VITAMINS IN FAVISM PATIENTS IN BASRAH GOVERNORATE -IRAQ

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ABSTARCT: The presence of various biochemical indications leads to get the good health for human but abundance of these markers with lower or higher concentrations than normal level leads to biochemical disorders . Favism disease is one of multi-disorders take place in the red blood cells . The study results showed presence of glucose-6- phosphate dehydrogenase (G6PD) in very low concentrations in patients depending on sex factor , therefore highly significant decreasing ($p<0.0001$) was recorded in patients compared with control group . Also no significance difference was found ($p>0.05$) in Glutamic Pyruvic acid Transferase (GPT) but catalase (CAT) enzyme recorded a high significant lowering ($p<0.0001$) in favism patients compared with healthy groups . Non-enzymatic antioxidant are represented by vitamins E and A showed highly significance difference ($p<0.0001$) between G6PD patients and control groups .

***Keywords*:** Favism disease, G6PD deficiency , catalase , GPT , vitamins E and A .

INTRODUCTION

Favism disease is a risk biochemical metabolic disorder happens in human being body because of presence of glucose-6- phosphate dehydrogenase (G6PD) enzyme in very low concentration in the living cell . G6PD is responsible for the complete work of red blood cells . The necessity of this enzyme appears clearly in pathway of pentose – phosphate pathway abundant in carbohydrates metabolism . The G6PD transforms glucose-6-phosphate into 6-phosphogluconate and this biochemical reaction takes place in presence of reducing ability represented by NADP+ [1,2] . In red blood cells , G6PD catalyzesfull oxidative stress reactions and

this biochemical statement takes place in presence of the gene which has ability for the activity and action of G6PD . it is Known that this enzyme has important role

in carbohydrates , amino acids , proteins and fats which have biochemical relationship with some non-enzymatic antioxidants markers like vitamins E and A and some types of proteins such as albumin and billirubin [3,4] . The deficiency in level value of G6PD is considered as an indication healthy complication affects some enzymatic and non-enzymatic antioxidants markers. Favism disease is one these complication that cause different disorders in whole metabolism especially carbohydrates pathways and this disease leads to various differences in the level enzymes , blood proteins vitamins , trace elements and lipid profile[5,6] . The biochemical disorder in deoxyribonucleic acid (DNA) especially in the gene of G6PD and also clinical disorders take place because abundance of different mutations so this biochemical case leads to lack in G6PD level in human being [7,8] . The genetic mutations happen because of biochemical disorder in X-chromosome leads to high deficiency in G6PD level especially in males .Also many enzymes lose some of their activity when G6PD is in low level [9] .The complication of favism disease is biochemically correlated with hemolytic anemia since this disease also takes place in red blood cells leading to fast destruction of these cells in individuals body. and the deficiency in G6PD causes various diseases like jaundice and hematuria which effect biochemical functions of liver and kidney [10]. The present study was carried out for estimation of oxidative stress for G6PD deficiency and some enzymes such as GPT and CAT and vitamins E and A.

Materials and methods

All blood samples were collected from favism patients and healthy individuals from general Abu Al-khaseebhospital at Basrah Governorate in Iraq . The number of patients was 100 (65 males and 35 females) and healthy persons as control group was 75 (45 males and 30 females) .All patients and healthy

Human have age ranged between(1-66 years) and all biochemical markers were measured according to type of sex . Also all clinical tests were insured such as sex , presence or absence other disease ,

marriage case , smoking status and family history .

Blood samples preparation

5 ml of Venous blood was gotten from (patients and healthy) . The blood samples were clotted and centrifugation process was established for 10 minutes with to 4000 rpm after that the blood sera were kept in 20°C until the usage day whereas residual blood was collected in tubes containing heparin .

Also the blood samples were centrifuged with to 3000 rpm for 10 minutes to get plasma whereas the enduring erythrocytes were washed quietly by 9%w/v sodium chloride solution and they are lyasated by using double deionized water with ratio (1:1 v/v)[11] .

Evalution and Assay of G6PD, GPT, and CAT enzymes in the blood

The G6PD levels were investigated and estimated in the whole blood by using automated procedure [12] and GPT concentrations in blood sera were estimated [13] . Also CAT enzyme wasdeterminedspectrophotometrically in blood serum [14,15] .

Determination of vitamins A and E in the blood

The concentrations of vitamin A and E were estimated by ELISA technique in blood serum of favism patients and healthy individuals [16,17] .

Statistical analysis

All results of the study were established and expressed by measure of mean ±standard deviation (SD) . Also the whole results data were analyzed by using a statistical method according to variance univariate analysis . Statistical programme of social science (SPSS,V23) was used to calculate the significant differences among various groups of patients and healthy individuals . The coefficient of correlation regression (r) was carried out to recognize between means of patients groups and control groups . The p-value was corresponded for less than 0.05 for the lowest significant limit .

RESULTS

The high risk for G6PD deficiency disease result from great lack of this biochemical enzyme in the red blood cells . It is known that glucose-6-phosphate dehydrogenase catalyzes pentose phosphate pathway in carbohydrates metabolism , so any biological and chemical disorder in this enzyme will lead to different complications in the physiological role belonging to red blood cells . Therefore investigation and estimation of levels of G6PD in whole blood is

considered as required status for following the healthy or patients individuals [18,19,10] . Table (1)indicates the concentrations of G6PD which were recorded in favism patients and healthy groups depending on sex factor (male and female) . The levels of this enzyme were measured to be equal to 0.66 and 0.66 mU\gHb in favism patients according to males and females respectively . Also the G6PD concentrations were estimated in values equal to 11.94 and 11.01 mU\gHb in healthy individuals (control group) in males and females respectively..

Table (1) :Concentrations of G6PD enzyme in whole blood in favism patients and control group according to sex factor

Groups	***Sex***	***G6PD (mU/gHb)***
Control	Male .no.=45	11.94±3.74
	Female .no.=30	11.01±2.61
Favism Patient	Male .no.=65	0.92± 0.79***
	Female.no.=35	0.66±0.57***

Values were expressed as mean ± SD.

* The level of significance between Patients and control groups

***P < 0.0001

The levels of Glutamic acid - pyruvic acid transaminase (GPT) enzyme in G6PD deficiency disease are illustrated in table (2) . The concentrations of GPT were 28.03 U/L in males patients and 28.49 U/L in females patients whereas found to be 26.22 U/L and 26.80 U/L in control groups.

Table (2) : Concentrations of GPT and CAT enzymes in blood serum in favism patients and control group in according to sex factor .

Groups	***Sex***	***CAT(K/ml)***	***GPT(U/L)***
Control	Male .no.= 45	1.71±0.021	26.22±7.17
	Female .no.= 30	1.74±0.024	26.80±5.44
Favism Patient	Male. no.= 65	0.16±0.011***	28.03±7.41#NS
	Female .no.=35	1.74±0.024***	28.49±6.88#NS

Values were expressed as mean ± SD.

*The level of significance between males and females in patients and control groups in CAT

[#] The level of significance between males and females in patients and control groups in GPT

[***] P < 0.0001 , [#]P > 0.05 and NS- Non significant

Concerning CAT , it was noticed that the levels of this enzymatic antioxidant was equal to 0.16 K/ml in males and 1.74 K/ml in females in favism patients whereas the concentrations of this enzyme in healthy groups were determined to be 1.71 and 1.74 K/ml in males and females respectively .

Vitamins A and E are considered as non-enzymatic antioxidants have important chemical roles in the living cell [(20)] . Figure (1) represents the standard calibration curve of vitamin A by using the concentrations of (0.156 , 0.312 , 0.625 , 1.25 , 2.5 and 10.5 ng/ml) then the optical density (O.D) was measured for each concentration .

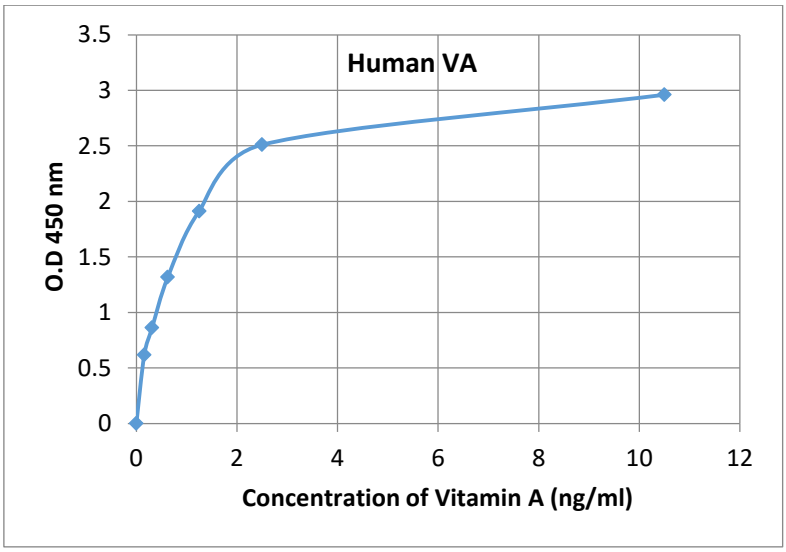

Figure (1) : Standard calibration curve of vitamin A

Figure (2) shows the standard calibration curve of vitamin E for different concentrations were represented by(1.56 , 3.13 , 6.25, 12.5, 25.0 , 50.0 and 100 µg/ml) and recored the optical densities at the same time for these concentration the same time the optical densities were recorded for these concentrations .

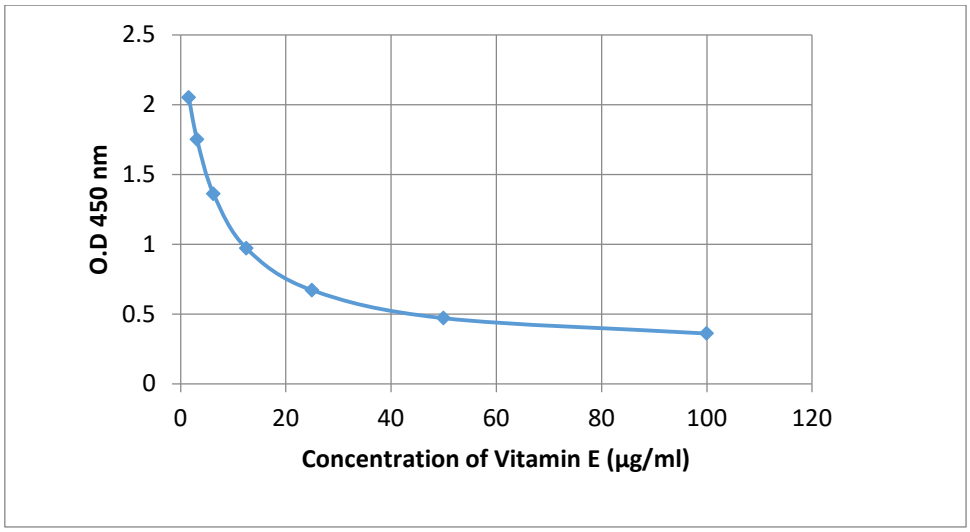

Figure (2) :Standard calibration curve of vitamin E

Vitamin A concentrations in blood serum of patients and control groups were indicated in table (3) . The levels of this antioxidant vitamin were found to be 4.01and 3.70 µg/ml in favism patients according to sex factor (males and females) respectively , whereas in control groups , recorded to be 7.52 µg/ml in males and 7.77 µg/ml in females .

Concerning vitamin E concentrations , it was noticed that the levels of this vitamin differed were from vitamin . The levels values of vitamin E were found to be 77.62 and 74.65ng/ml in males and females respectively in favism patients , but the concentrations were measured to be in values equal to 104.72 ng/ml in males and 107.41 ng/ml in females in healthy individuals groups.

Table (3) : Concentration of vitamins A and E in blood serum of favism patients according to sex factor

Group	***Sex***	***Vit. A(ng/ml)***	***Vit. E (µg/ml)***
Control	Male .no.=45	7.52±1.02	104.72±11.49
	Female no.=30	7.77±0.93	107.41±11.88
Favism Patient	Male .no.=65	4.01±1.06***	77.62±9.41***
	Female .no=35	3.70±1.07***	74.65±10.33***

Values were expressed as mean ± SD.

*The level of significance between patients and control

***P<0.0001

DISCUSSION

Favism disease is a dangerous and complex disorder take place in red blood cells because of the great deficiency in glucose-6-phosphate dehydrogenase (G6PD) enzyme . So the patients of favism disease have many various healthy problems such as biochemical disorders in enzymatic antioxidants are responsible for complementary of biochemical pathways especially in carbohydrates and lipids metabolism [21] . Also G6PD deficiency makes multi-disorders in levels of different vitamins , lipid profile , blood proteins , trace elements , uric acid and malondialdehyde . Therefore the concentrations of these biochemical components will change depending on disease severity, age and sex belonging to favism patients[22] .

Table (1) represents the various concentrations for G6PD in both favism patients and healthy individuals (control groups) . It was showed low of G6PD in favism patient compared with control group for both male and females Therefore a highly significance difference ($p<0.0001$) was measured between favism patients and control groups in males and females . The attendance of G6PD high significant decreas indicates a risk pathogenic status represented by favism disease so this case leads to decomposition and destruction of red blood cells in human body[23] . As a result , the high lack in G6PD enzyme levels will lead to weaken the physiological and chemical effects of red cells of blood and this enduring status will certainly lead healthy and biochemical dangersespecially in lipids content , enzymes concentrations , vitamins levels , blood proteins concentrations and some trace element[24] .

The values of GPT enzyme indicated that no significance difference ($p>0.05$) between favism patients and control groups for both males and females as in table (2) .This status may be explained as the G6PD deficiency didn't effect somewhat on levels of GPT enzyme especially in males . Also from table (2) , we were noticed that highly significanct decrease was recorded ($p<0.0001$) in concentration of CAT enzymein favism patients compared with control groups in males and females together . So that CAT levels in blood serum was considered as important indicator for diagnosis of severity of G6PD deficiency disease [25,26] .The biochemical role of catalase enzyme is catalyzing reaction of decomposition of hydrogen peroxide to water and oxygen in the living cell .Therefore this enzyme is very important for protection the cell from oxidative risk by reactive oxygen species [27] .

Table (3) reveal to the concentration of vitamin A in favism patient and control group and the result show that high significant ($p<0.0001$) value when compared with control group in both male and female . antioxidant is an biochemical marker is correlated with favism disease then vitamin A is considered as benefit biochemical indicter to diagnose the severity of G6PD deficiency because of presenceof biochemical relationship between oxidative stress by vitamin A with favism disease [17].

High significance difference was found in vitamin E concentrations ($p<0.0001$) between favism patients and healthy control groups in both males and females then levels of vitamin E in blood sera are considered as a necessary indication explains the oxidative stress of this vitamin in physiological action belonging erythrocytes . Therefore there is an important relationship between vitamin E concentrations in blood serum with favism disease[28] .

CONCLUSIONS

The present study was established to investigate and estimate some enzymatic and non-enzymatic antioxidants which have a biochemical relationship with G6PD deficiency disease . It was found that G6PD concentrations significant decrease in favism patients . Catalase as an enzymatic antioxidant proved the biochemical correlation between this enzyme with G6PD deficiency therefore CAT has important action for investigation of the severity of favism disease . GPT had no effect with lack of G6PD enzyme in both males and females .Non-enzymatic antioxidants represented by vitamin A and E indicated that a great relationship with G6PD deficiency in blood serum for males and females patients . As a conclusion , catalase , vitamin A and E are considered important markers for diagnosis of favism disease severity .

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تأثيرنقص انزيم لكلوكوز -6- فوسفات ديهايدروجينيز على بعض الانزيمات والفيتامينات في مرضى فقرالدم الباقلائي في محافظة البصرة – العراق

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البصرة – العراق

الملخص :ان وجود المؤشرات الكيموحياتية المختلفة بتراكيز طبيعية يشير الى الحالة الطبيعية للانسان ولكن وجود هذه المؤشرات بتراكيز اعلى او اقل من المستوى الطبيعي يؤدي الى الاضطرابات الكيموحياتية .يعتبر مرض فقر الدم الباقلائي هو واحد من الاضطرابات المتعددة التي تحدث في كريات الدم الحمر . وقد اظهرت النتائج الدراسة اظهرت وجود كلوكوز – 6 فوسفات ديهايدروجينيز بتراكيز واطئة جداً في المرضى اعتماداً على عامل الجنس ، وعليه فأن انخفاض معنوياً عالياً ($p<0.0001$) قد لوحظ في المرضى مقارنةً بمجموعة السيطرة . بينما لم يوجد هناك فرق معنوي ($p>0.05$) في حامض كلوتاميك –بايروفيك ترانسفيريز ولكن انزيم كاتليز سجل انخفاضاً معنوياً عالياً ($p<0.0001$) في مرضى فقر الدم الباقلائي مقارنةً بمجموعة الاصحاء . مضادات الاكسدة غير الانزيمية المتمثلة بفيتاميني A و E أظهرت فرقاً معنوياً عالياً ($p<0.0001$) بين مرضى G6PD مقارنة بمجموعة السيطرة .

الكلمات المفتاحية : مرض فقر الدم الباقلائي ، نقص G6PD ، كتاليز ، GPT ، فيتامينات A , E